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Another modification of the Scholander – Roughton technique: Nitrogen determination in fish blood

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Certain difficulties are encumbered when one wishes to do gasometric determination of blood gases in fish blood. The clogging problem makes it necessary to have a high degree of acidity in the final blood mixture for breaking up the proteins. The present method was developed during an investigation of nitrogen deposition in the swimbladder of whitefish and other salmonidae (Sundnes, Enns & Scholander, 1958). Part of the method for oxygen determination in fish blood by Scholander & van Dam (1956) was modified and combined with a part of the method for nitrogen determination by Edwards, Scholander & Roughton (1943). The present modification has been tested on blood from Gadide and Salmonide fishes. Details of the analysis proceedings are given below.

APPARATUS

The syringe analyzer and the calibrated pipette, with a capacity of about 40 mm^3, are described by Scholander & Roughton (1942). The pipette is calibrated to deliver 2 to 3 times the volume used in the general methods. The strong acid used in the method produces a rapid CO_2 evolution, which makes it necessary to close the analyzer with the wire clip described by Scholander & van Dam (loc. sit.).

REAGENTS

1. Aerated distilled water.
2. Isotonic saline. Dissolve 11.7 gm NaCl in 1 liter of water (= 0.20 M).
3. Caprylic alcohol.
4. Acid sulfate solution. Dissolve 30 gm Na_2SO_4 anhydrous in 100 ml water and add 5 ml concentrated H_2SO_4.
5. Ferricyanide solution. Dissolve 12.5 gm $K_3Fe(CN)_6$, 6 gm $KHCO_3$ and 0.5 gm saponin in 50 ml water.
6. Hyposulphite solution. 4 gm of a mixture of sodiumhyposulphite ($Na_2S_2O_4$), 10 parts, and sodium anthraquinone — β — sulfonate, 1 part, are added to 10 cc of 20 per cent KOH. This solution should be made in a 10 cc syringe with the least possible contamination from air.

Reagents 2, 3, 4 and 5 are in 2 cc syringes and reagents 1 and 6 may best be stored in 10 cc syringes.

In this method the ferricyanide solution is used while reagents 1 to 5 also are used in oxygen analyses of Salmonide blood.

PROCEDURE

The analyzer and the blood pipette should be cleaned with dichromate H_2SO_4 solution and then rinsed with water. Dry the pipette by suction. Rinse the analyzer three times with the acid sulfate solution. Hold the syringe vertically and fill acid sulfate solution to the mark of the cup. Draw the solution into the capillary, rinse the cup with saline, and empty. Place a drop of caprylic alcohol in the bottom of the cup and expel trapped air bubbles. Fill the blood pipette with two or three times the ordinary amount of blood for oxygen analyses (depending upon the amount of dissolved gas in the blood). Transfer the sample into the analyzer the usual way. Seal the capillary by drawing in caprylic alcohol to the zero mark, and remove the rest by suction. Fill the cup with the ferricyanide solution.

The metal plug should be placed in the bottom of the cup, but not closing the capillary completely. Draw the ferricyanide solution into the barrel, and close the capillary immediately by seating the plug. Shake the analyzer horizontally, and at short intervals loosen the plunger in the syringe to equalize the pressure. In two minutes the gas evolution is ended.

The gas pressure in the syringe has to be adjusted to the outside pressure with the plunger. Remove the plug carefully, and keep the liquid seal in the capillary by manipulating the plunger. Attach the rubber cup and add the hyposulphite solution without trapping air in the syringe. Draw some hyposulphite gently into the barrel, letting the plunger up during absorption.

Empty the rubber and syringe cups by suction and remove the rubber cup. Fill the syringe cup with water and draw three-fourths of it down over the bubble. If necessary clean the upper part of the capillary by twirling a fine stainless steel wire in it. Push the bubble very gently into the clean capillary and temperature equilibrate the analyzer in water of room temperature for half a minute. Dry the analyzer by light wiping, and read the volume of the bubble (v).

The amount of N_2 in the reagent is determined by running through the experimental procedure, without introduction of blood. The first determination of the blank will give some difficulties due to the rapid CO_2 evolution.

The gas evolution starts immediately when the bicarbonate comes in contact with the acid. In the blood analyses the gas evolution is much more damped by the blood.

The nitrogen content in volume per cent equals $(V-c) \times f/a$ where c is the blank correction for nitrogen in the reagents, f is the STP correction, and a is the volume of the blood used in the pipette (i. e. 1, 2 or 3).

For Gadide fish the procedure for oxygen determination described by Scholander & van Dam (loc. sit.) can be followed until the CO_2 evolution. From that point the present described modification is followed.

RESULTS

In the present method the nitrogen content of the blood was varied by altering the partial pressure of the nitrogen. The blood was saturated in a slow rotating syringe in a water bath. To vary the partial pressure the gas in the syringe was compressed with the plunger. Duplicate and tripple analyses in Salmonide blood from several specimens agreed within $\pm$ 0.02 volume per cent.

The nitrogen content (i. e. the unabsorbable part of air) in distilled water, saturated with air at different pressure was determined by the present modification. The measured values were compared with calculated data from Handbook of Chemistry and Physics (1956—1957) in Table 1. Analyses (with the fish blood reagents) of distilled water, agreed within $\pm$ 0.03 volume per cent of the calculated values.

Table 1

Determination of nitrogen in distilled water saturated with air containing 79,01 % nitrogen compared with calculated values. The volumes are reduced to dry roulumes at 0° C and 760 mm Hg.

P. (air total)	Temp.	Vol. % measured	Vol. % calculated
753 mm	7.6° C	1.49	1.52
754 «	4.8° «	1.64	1.63
759 «	4.8° «	1.67	1.64
1163 «	3.6° «	2.59	2.59
1186 «	4.0° «	2.64	2.61

The nitrogen capacity of Salmonide blood follows Henry's law as previously described by Sundnes, Enns & Scholander (loc. sit.).

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LITERATURE CITED

EDWARDS, G. A., SCHOLANDER, P. F. & ROUGHTON, F. J. W. (1943). Micro gasometric estimation of the blodd gases. III Nitrogen. *J. biol. Chem.* 148, 565–71.

Handbook of Chemistry and Physics (1956–1957) 38th edition. Chemical Rubber Publishing Co., Cleveland, Ohio.

SCHOLANDER, P. F. & VAN DAM, H. (1956). Micro gasometric determination of oxygen in fish blood. *J. cell. comp. Physiol.* 48, 529–32.

SCHOLANDER, P. F. & ROUGHTON, F. J. W. (1942). A simple microgasometric method of estimating carbon monoxid in blood. *J. industr. Hyg.* 24, 218–21.

SUNDNES, G., ENNS, T. & SCHOLANDER, P. F. (1958). Gas secretion in fishes lacking rete minabile. *J. exp. Biol.* 35, 671–76.